

THE RELATIONS OF BURSARIA TO FOOD.

I. SELECTION IN FEEDING AND IN EXTRUSION

BY
ELMER J. LUND

A DISSERTATION SUBMITTED TO THE BOARD OF
UNIVERSITY STUDIES OF THE JOHNS
HOPKINS UNIVERSITY
IN CONFORMITY WITH THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF
PHILOSOPHY

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THE RELATIONS OF BURSARIA TO FOOD

I. SELECTION IN FEEDING AND IN EXTRUSION

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INTRODUCTION

While studying the phenomena of regeneration and structural regulation in *Bursaria*, it became desirable to know something of the relations of this organism to its food and of the processes which the solid food material undergoes in its passage into and through the cell and in the elimination of residues. This paper aims to present the first part of these observations and to make a general survey of the relations of this organism to food, leading up to a more detailed study of these and certain other problems of the cell as found in this unicellular animal.

Bursaria was found to be much more favorable for the investigation of these phenomena than the smaller infusoria such as *Paramecium*. No study has heretofore been made of the relations of *Bursaria* to food, so that the facts herein presented are new. The present investigation attempts not only to determine qualitatively whether the relations to food are similar to those more or less known for *Paramecium*, *Stentor*, *Vorticella* and other infusoria, but more particularly to work out and express these relations in a more quantitative way than has been done heretofore. It was found that certain kinds of experimental tests such as those on the rate of digestion, could be made upon this form, which it would have been difficult or impossible to carry out on smaller unicellular organisms. Its very large size offers a singular opportunity for easy manipulation in many kinds of work. When in a clear medium it is readily visible to the naked eye at a distance of six or eight feet and individuals may be transferred singly with a pipette without the aid of any magnifying instruments.

Bursaria occurs not infrequently in cultures brought from ponds in the vicinity of Baltimore, though it is less common than many other Protozoa. It can readily be cultivated in large culture dishes in the laboratory. In this way I have had abundant material at my disposal for many months. The method of cultivation has been simply the inoculation of an infusion of timothy hay in tap water from the wild culture; by several inoculations at different times one usually succeeds in obtaining large numbers. Since the food of this organism is not bacteria,

so far as I have yet observed, but a variety of other ciliates, flagellates and rhizopods, it is difficult to find a culture medium which can be readily manipulated, and hence pure line cultures can not be obtained so readily as of a form like *Paramecium*. The problem of pure line cultivation of this organism will not be dealt with in this paper. The material for use in the following experiments has all been obtained from mixed or 'wild' cultures, though the reinoculations from the single parent culture brought into the laboratory seven months ago resulted in a small number of pure lines living side by side in the cultures.

It is, in fact, preferable in some ways to use material from such wild cultures for the kind of experiments to be considered in this paper.

Even without the aid of pure cultures or the application of statistical methods to wild cultures it soon became apparent that there are actually at least two very distinct races of *Bursaria* which differ in several diverse characters, physiological as well as morphological.¹ One form, which under certain food conditions has a tail, has been used exclusively in these experiments, since the other form, collected at the same time and lacking a tail, died out early in the experiments.

I have observed the following organisms to be eaten and digested by *Bursaria*: *Chilomonas*, *Colpidium colpoda*, *Vorticella* and some of its relatives, *Oxytricha*, *Stylonychia*, *Arcella*, *Stentor*, *Paramecium*, *Stephanodiscus*, and some kinds of rotifers. Only once have I observed bacteria to be eaten, and that time in the form of zoogloea. It is, however, certain that bacteria form only a small part, if any, of the usual diet of this organism. The smaller ciliates, flagellates and rhizopods are the favorite article of food. The larger organisms, such as *Stentor*, are seldom successfully captured. *Paramecium* is quite commonly eaten, though *Bursaria* does not seem to thrive well on this food. Occasionally rotifers are eaten and it was observed on several occasions that these may remain alive within the vacuole

¹ I have been unable to find reference in the literature to more than one form of *Bursaria*. A consideration of the problems connected with the existence of diverse 'races' of *Bursaria* will be left for a later time.

for as long as five hours before they are killed. It is, however, plainly evident when one follows the development of wild cultures from day to day that some forms are eaten in greater numbers than others and if the smaller forms, such as *Colpidium*, *Vorticella* and *Arcella*, are present in abundance along with such forms as *Paramecium*, *Stentor* and *Stylonychia*, the former kinds serve exclusively as food for *Bursaria* while the latter are rarely eaten. When the cycle of development of the culture comes to the stage where, for example, *Paramecium* is in superabundance, then the body of *Bursaria* may be more or less filled with *Paramecia*. In contrast to the above mentioned forms, *Spirostomum ambiguum* was always rejected. It was often seen to be taken into the oral pouch but invariably was thrown out again, while *Paramecia* present in the same culture were readily eaten by the same *Bursaria* individuals at the time of the observations. This is the only case where *Bursaria* was seen definitely to discriminate between two different forms of Protozoa.

By simple methods of observation like the above, it would be impossible to determine just what the principle and the factors are that determine whether *Bursaria* will feed on only one or several or all of these forms if they be present in all the cultures simultaneously, which of course they often are. It is with the object of elucidating these and certain related questions that the following comparatively simple experiments have been performed, by limiting and determining to a high degree the conditions under which this organism will react to food.

ACTION OF THE STRUCTURAL MECHANISM FOR FEEDING AND THE SELECTION OF FOODS

An account of the food relations of *Bursaria* requires us to examine in some detail the objective processes involved in feeding; these are very striking. The highly developed oral apparatus with its large cilia, when in operation during the feeding process, may easily be observed. When the organisms are fed on such substances as yolk or starch they usually sooner or later become quiet for a time, and settle to the bottom of the dish or stick to the surface film of the water, then they may be

observed under a high power of a binocular. Granular substances of different chemical or physical properties may be placed in the medium and the path of each individual particle may be easily observed. Mixtures of these substances may also be made and the paths of the different kinds of particles may be determined.

The different paths of particles which come into varying relations with the organism are shown by the arrows in the outline drawings of figure 1. The paths of the arrows are correct representations of the paths taken by the different kinds of particles. In general the paths taken by particles may be distinguished according to the following outline:

1. Paths of rejection

- a. Path of total rejection, arrows A
- b. Path of rejection of larger particles taken into the oral apparatus, arrows B
- c. Paths of rejection of smaller particles taken into the oral apparatus, arrows C_1 and C_2 .

These paths may also be slightly modified by a combination of the avoiding reaction with the different rejection reactions.

2. Path of acceptance of large and small particles (large arrows D)

Path A is taken by those particles which under conditions hereafter to be considered (p. 29) never enter the oral apparatus and are only drawn towards the body by the current; for example, very toxic particles of yolk. Path B is always taken by those particles which are too large to pass out by way of path C_1 and C_2 and must be passed back to the exterior by the same way as they were taken in, in order for the organism to get rid of them at all. This may be illustrated by the larger properly treated grains of hard boiled yolk. The path represented by the arrows C_1 and C_2 has considerable range of variation in part of its course. It may be illustrated by cornstarch grains; these are of convenient size. The variations in the course of these particles may be divided roughly into two main divisions; some follow the dotted arrows C_2 and never directly retrace any

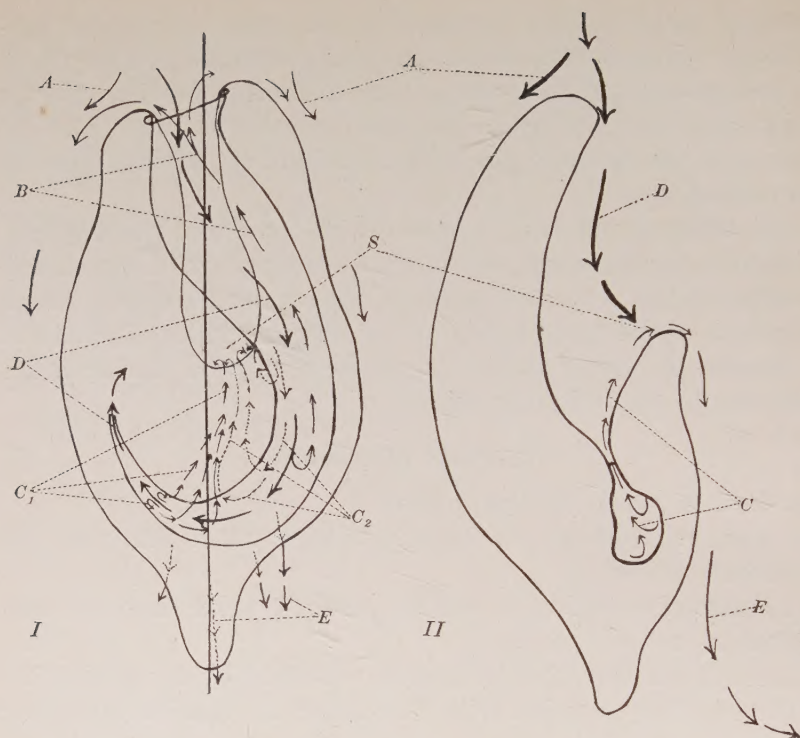


Fig. 1 *I*, Outline drawing from dorsal side of *Bursaria*, to show position of the oral pouch in the body, and the paths of variously rejected and accepted particles. *A*, path of total rejection; *B*, path of rejection of large particles which are too large to pass out by way of the oral sinus, *S*. *C*₁ and *C*₂, paths of rejection of small particles; these pass out by way of the oral sinus, *S*; *D*, path of acceptance. *II*, Outline drawing of sagittal section, in the plane through the body represented by the straight line through *I*. *A*, path of total rejection; *D*, path of entrance of all particles taken into the oral pouch, same as first part of path *D*, *I*. *C*, path of rejection of small particles, same as *C*₁ and *C*₂ of *I*. *E*, *I* the same as *E* *II*, direction of rejected particles, *C*, *I*, and *C*₁, *C*₂, *II*.

part of their former path; some pass up into the proximal end of the oral pouch but are rejected and returned to the outside by way of the continuous arrows *C*₁. All the paths of rejection under *C*₁ and *C*₂ converge and lead to the exterior by way of the oral sinus, figure 1, *I* and *II* *S*.; they then pass backward under the posterior ventral side, as shown by arrows *E*. There is but one path of acceptance for both large and small particles.

Figure 1, *II*, is a sagittal section through the body in the plane indicated by the straight line through figure 1, *I*; it shows the path of total rejection, arrows A, the path of entrance (by heavy arrows D) and the path of rejection of smaller particles (by arrows C).

At the point of entrance into the endoplasm the transport of the accepted particles is brought about not alone by the cilia but also if not exclusively, in the case of larger particles, by a peristaltic wave in the wall of the oral cavity behind the particle pushing it into the body.

FORMATION OF THE VACUOLE AND THE ELIMINATION OF RESIDUES

The vacuoles when formed always contain some liquid, though at times the amount may be very small. The size and shape of the vacuoles varies greatly and depends upon the kind of food eaten, and upon many other conditions, as will be shown. Often the food forms large irregular masses, which in the case of fresh yolk may so completely fill the body that after a half-hour or more of feeding the dorsal side of the body cortex is burst open and the food mass is extruded. The opening then closes and the organism again assumes its usual shape. The rate of formation of the vacuoles is intimately bound up with the same complex conditions which determine their size and shape. The circulation of the vacuoles in Bursaria is not reducible to any definite order, such as has been shown to exist more or less definitely in *Paramecium*, by Metalnikow ('12) and others, and in *Carchesium* by Greenwood ('94). The vacuoles often become lodged in one place and there digestion is completed. This may often be seen in cases where fat-extracted yolk particles become lodged in the tail. During digestion and resorption the large vacuoles usually become smaller and any residual contents are finally extruded. The residues are always extruded from a small area on the mid-dorsal side of the body of the organism. This may readily be demonstrated by feeding the animals chinese ink. The changes which take place in the food vacuole from its formation to its disappearance will be considered in detail in a later paper.

MEASUREMENT OF THE AMOUNT OF FOOD EATEN AND METHOD
OF EXPERIMENTATION

In order to express quantitatively the relations of *Bursaria* to food, it is necessary to obtain a reliable method for measuring the amount of food taken in a given length of time, under given conditions. The unit of volume employed was that of one grain of fresh hard-boiled yolk of hen's egg. The eggs were boiled fifteen minutes. These grains are readily eaten by *Bursaria* and may be obtained of an approximately uniform size. It is necessary to deal with suspensions of such grains, having a uniform concentration (that is, containing the same number of grains to a given volume). For this purpose stock suspensions were made up on successive days from yolk of the same egg: these were made uniform by making them up in vials of the same size and comparing each with a standard concentration kept in a vial of the same size. Various known grades of concentration were then made up by adding a known volume of the stock suspension to 5 cc. of water in a stender dish of 8 cc. capacity. This procedure was found to be sufficiently accurate to avoid the introduction of any observable variation in the amount of yolk eaten in a measured period of time (page 20.²) Uniformity in the size of the yolk grains was obtained by repeatedly washing the fresh hard boiled yolk crystals in distilled or tap water and decanting, until the suspension when left to settle leaves a clear supernatant liquid. The smaller grains remain in suspension a little longer than the larger ones and thus may be removed by decantation. Uniformity in size is still further obtained by drawing off the grains from the same level in the clear suspension with a pipette. Some eggs have yolk crystals of more uniform size than others, so that only the eggs best in this respect have been used.

² In most of the experiments it was necessary to make up only a single stock suspension, since the animals were fed only once and all the feeding was carried out at the same time. In the case of experiments which required the feeding of yolk on more than one day, however, this standard concentration had likewise to be made up anew each day by comparison with that of the day before.

The uniformity in size of the yolk grains is of course of paramount importance in many of the experiments and for some of the conclusions which will be drawn from them. In order that the degree of uniformity might be tested and indicated quantitatively, a large number of measurements of grains of the prepared yolk suspension were made at different times by means of a stage micrometer. The following shows a typical result of one set of these measurements: 105 grains were measured and the numbers divided at random into three sets of 35 each and the average of the diameters taken. These gave respectively 0.0890, 0.0906, and 0.0837 mm. The range of variation of the diameter was from 0.060 to 0.130 mm. The distribution of the variations are shown by the following figures:

Diameter of grain mm.....	0.06	0.07	0.08	0.09	0.10	0.11	0.12	0.13
Frequency.....	12	19	16	14	20	13	6	5

By thus being able to obtain a very constant average diameter of a comparatively small number (30 to 50 grains) the errors introduced by the individual variation in size, which in the above example is about as $\frac{1}{2}$ is to 1, is largely eliminated. In order to remove the objection to experimental results based on the volume of granules of varying size; a large number of individuals (20 to 100, depending upon the purpose of the experiment) were used in each experiment and the number of grains eaten was counted; furthermore the experiments were always repeated whenever there could be any doubt as to the validity or significance of the results. Hence, as will be shown later, the lack of strict individual uniformity of the unit volume is corrected (a) by the fact that the average size of the yolk grain is practically constant, (b) by using a large number of individuals in each experiment, and (c) by repeating the experiment.

Thus having the form, weight and volume of the units of food eaten made practically constant, we may vary one of their properties—as for example, their chemical nature—by letting them adsorb different kinds of toxic and non-toxic substances which are diffusible or non-diffusible in the native medium, tap or distilled water. We may therefore test the responses to variations

in this one property—namely, the chemical nature of the grain—and its effects.

An approximately constant medium was provided by using tap water. This precaution is important, for, as will be shown, the nature of the medium often affects or determines the kind of results which are obtained. Distilled water was also used but it was found that this extra precaution was not necessary in most of the experiments, and since distilled water is toxic if the organisms are left in it too long or the change is too rapid, it could not have been used in many of the experiments, even if it had been otherwise desirable to do so.

The organisms were starved in 400 cc. of tap water for eighteen to twenty-four hours previous to each experiment. At the end of this time they were free from food and residues. Thus an optically clear, active and perfectly normal cell was obtained with which to begin work in all the experiments where uniformity in this respect was desired. All the factors with which we are dealing except the 'physiological states' of the organisms themselves are known and uniform to within narrow limits, while the one of which we wish to test the effects can be controlled and varied.

INTERNAL RELATIONS AFFECTING THE FEEDING PROCESS

1. The relation of the physiological state of the organism to the feeding process

By the words 'physiological state' is here meant the condition as a whole, of the equilibria in the physical and chemical reaction system, the cell, at a certain time in the duration of its existence.³

This condition or state is to be thought of as being limited to the space which the organism occupies, or is, in other words, internal. However, it is obviously absurd for anyone to attempt

³ This definition is justified because in so far as the facts are at present known, this is the only kind of system with which we have to deal in the cell, and therefore in the present state of knowledge the only logical universal assumption for experimental purposes is to define 'physiological states' in terms of such known systems, until the universality of the assumption is disproved.

a definite and strict separation of the internal and external of any living organism, and especially is this true of the cell. Yet for purposes of presentation, this becomes highly convenient, and it is only for this purpose that the above rough distinction is made here. *When all external conditions are made the same in two experiments which nevertheless give different results, the differences must be attributed to different conditions within the organism,* and it is, as a rule, only in this way that different physiological states are at present practically perceptible.

Differences in physiological state in unicellular animals are made evident most readily in the relations to food, as may be seen from the work of Metalnikow ('12) on *Paramecium* and by Schaeffer ('10) on *Stentor*.

Bursaria affords most excellent material for the elucidation of the relation of these dynamic states to the feeding process and of the fact that this relation changes while the external conditions remain constant. These facts are brought out in the following experiments by using both single individuals, and large numbers of individuals collectively, at the same time, and analyzing the results.

The total quantity eaten and the rate of feeding. Table 1 gives the results of a typical experiment designed to show the difference in the total quantity of food eaten and also the difference in the rate of feeding of *Bursaria* from different cultures.

Material from two different cultures, *A* and *B*, was starved twenty-four hours in each of two dishes containing 400 cc. of tap water. 1 cc. of a fresh hard boiled yolk suspension was placed in each of 16 stender dishes of 8 cc. capacity; 5 cc. of tap water was then added to each. Thirty individuals from culture *A* were placed in each of 8 of the dishes. Alternately with these 8 sets from culture *A* were placed 8 sets of thirty individuals each from culture *B* in the other 8 dishes. At the end of the time intervals noted in the table, in each case, the contents (6.5 cc.) of one dish each of *A* and of *B* were transferred into a stender dish with 500 cc. of tap water. This stops the feeding. The individuals were then immediately picked out of these large dishes, placed in 8 cc. dishes and killed in Meves' fluid. The

counts of the number of grains contained in each individual were taken at the end of the experiment.

Table 1 shows (1) that *Bursariae* living in the two different cultures differ in the total amount of food eaten in the same length of time. In other cases, of course, individuals from diverse cultures will give identical results so far as feeding is concerned, while two or more different cultures may also differ to a greater extent than the above table shows. Moreover, the amount of food eaten by a given culture may vary at different times. The greater the length of time of feeding (within certain limits) the greater the total amount of food eaten. Not only does the total amount of food taken differ in the two cultures, but what is equally important, (2) the rate of feeding varies with organisms from different cultures. This was observed in numerous other experiments. Under some conditions the animals fill their bodies quickly, while at other times this takes place slowly; or only a small number of grains may be eaten.

The facts are shown most clearly by the curves *A* and *B*, figure 2, representing the number of grains of yolk (ordinates) eaten by the thirty individuals in successive periods of one-half minute (abscissae) throughout the time of the feeding process. Curve *A* is plotted from the results of culture *A* and curve *B* from those of culture *B*, in table 1. The immediate rapid rise of curve *A* shows that the rate of feeding of culture *A* during the first six successive periods of one-half minute each was about from five to twenty times as great as in any of the subsequent fifty-seven minute intervals. A similar high initial rate is also shown by curve *B* (culture *B*), but here the rise to the maximum was not so steep and the rate during the first six half-minute periods was only about from four to ten times the rate during the subsequent fifty-seven half-minute intervals.

In order to show more clearly that the results apply to the individuals taken separately as well as to the averages for all (i. e., to the cultures as a whole) the data may be arranged as in table 2. As this table shows, at the end of sixty minutes all but an insignificant number of animals from each culture had eaten yolk grains: hence, the difference in the amount and

TABLE 1

Showing the results of a comparison of the rate of feeding and the total number of grains of yolk eaten by eight sets of thirty individuals each from two different cultures, A and B

TIME FED IN MIN- UTES	CUL- TURE	NUMBER OF GRAINS OF FRESH YOLK EATEN BY EACH CELL																												TOTAL NUMBER OF GRAINS EATEN	AVERAGE NUM- BER OF GRAINS EATEN PER INDIVIDUAL	AVERAGE NUMBER OF GRAINS EATEN IN MINUTES = AVERAGE RATE FEEDING		
1	A	0	0	0	1	0	1	1	2	3	0	1	0	4	0	0	2	2	1	2	1	0	1	0	2	3	0	2	0	3	0	32	1.06	32.0
	B	0	0	1	0	0	0	1	0	0	0	1	0	0	1	2	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	10	0.33	10.0
1	A	1	2	1	0	1	0	4	0	6	1	1	4	6	0	2	1	0	1	6	2	4	4	8	6	3	0	4	2	0	70	2.33	38.0	
	B	0	0	0	1	4	0	1	0	1	2	0	2	0	3	0	3	0	0	1	1	0	0	1	0	0	2	0	0	4	25	0.83	15.0	
3	A	10	9	16	20	9	8	3	5	8	6	10	11	2	0	1	5	7	4	0	6	3	1	0	12	0	13	4	7	6	12	198	6.60	32.0
	B	0	1	3	1	0	1	0	1	0	3	0	0	2	2	2	4	0	7	4	0	2	1	3	6	4	3	2	0	4	58	1.93	7.0	
5	A	0	0	10	11	10	0	0	9	12	11	0	15	9	12	5	6	4	10	11	7	0	15	10	8	6	7	8	12	11	8	227	7.56	7.25
	B	3	3	4	6	1	4	3	2	3	4	0	2	0	1	0	3	0	2	1	0	3	2	4	0	2	6	2	5	1	4	71	2.37	3.25
10	A	9	24	7	10	8	4	9	5	16	6	11	12	3	10	8	4	8	3	0	0	3	16	3	14	16	4	7	0	12	9	241	8.03	1.4
	B	10	7	7	4	4	3	0	4	0	2	3	2	9	2	3	3	2	0	5	1	3	1	2	3	1	1	6	3	0	91	3.03	2.0	
20	A	14	10	10	15	3	7	10	13	7	4	11	7	0	7	3	23	10	9	15	12	18	12	15	5	14	19	8	9	2	292	9.73	2.55	
	B	1	1	6	8	1	0	4	3	6	3	0	1	6	1	1	9	9	3	8	0	8	3	4	9	0	0	0	2	114	3.80	1.15		
30	A	15	12	16	24	15	0	7	14	5	7	4	16	7	24	3	16	13	15	11	16	0	10	0	9	20	2	4	14	2	316	10.53	1.2	
	B	6	3	6	0	0	7	6	2	4	5	2	0	0	5	4	5	11	0	8	4	6	5	9	4	0	6	5	4	117	3.90	0.15		
60	A	16	0	11	5	8	19	0	8	20	20	5	23	6	12	12	15	29	9	11	14	6	13	22	13	24	15	16	11	0	19	381	12.70	1.08
	B	6	8	8	14	14	10	3	8	16	4	16	0	3	0	1	5	4	8	3	8	9	0	6	1	0	12	0	5	0	180	6.00	1.05	

rate of food taken by the two cultures was not due to some sporadic difference caused, for example, by a very high rate of feeding by a few individuals and no food eaten by others, but rather to a uniform difference between the sets of individuals from the two cultures. Therefore the results are typical for the individual as well as for the culture as a whole. Moreover, if we calculated the averages of *A* and *B* on the basis of those individuals alone which had one or more grains, the average of *A* would still be greatly in excess of that of *B*.



Fig. 2 Showing the rates of feeding by the two cultures *A* and *B*, curves *A* and *B*, respectively. Plotted from the results of table 1.

We may express the variation in the total quantity eaten by the standard deviation of each corresponding group of thirty individuals in *A* and *B*, as is done in the last column of table 2. The reciprocal of the standard deviation (σ) is a measure of the degree of uniformity among the individuals. It will be noted that there is an increase in the range of variation and the standard deviation with increase in the length of time of feeding; this means that the difference in physiological state among individuals of the same culture finds a fuller and more definite ex-

pression in the results of the experiment as the length of time of feeding is increased. The final total number of grains eaten when the time is long is then a more accurate index of the relation of the physiological state to the feeding process than if the time of feeding is short. The greater this difference in the total quantity of food eaten the greater is the difference in the physiological state of the different individuals. We, therefore, have in the amount of food eaten, if the length of time of feeding is long enough, a fairly good relative measure of the physiological state of the single individual and the differences in the physiological state between different individuals as regards their relation to food at that particular time.

2. Changes in the physiological state as shown by using the feeding process as an index

If change in the dynamic conditions of the cell, as regards the food relation, does occur, this should be observable by a change in the feeding process, and such is indeed the fact. This is shown in table 3. Material from cultures *C* and *D* was starved in tap water for twenty-four hours. Five active individuals were then picked out from each and tested individually. They were fed twenty minutes and each one was observed continuously during the experiment. The number of grains eaten and rejected and the time as called off by the observer were noted.⁴ In this way the time record of the relation of acceptance and rejection of food was obtained. The yolk concentration, temperature, and so forth were the same in all the tests.

As the table shows, yolk grains were at first rapidly eaten. At the end of the first few one-half minute intervals the action of the cilia was frequently reversed, thus rejecting the food after it had been taken into the oral apparatus. There was, therefore, a definite change from eating to the rejection of food by the feeding mechanism. This change was more rapid in general, in the individuals from culture *C* than in those from culture *D*.

⁴ I am indebted to Mr. K. S. Lashley for kindly aiding me in taking the records of this experiment.

TABLE 3

April 4: CULTURE C
Starved, tap, 24 hours

Tests on individuals

TIME MINUTES	1	1 1/2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SUM	
I	Accepted..	5	1	3	3	3	6	4	5	3	3	6	7	1	1	2	2	5	6	7	3	2	12●
	Rejected..				3	3	3	6	7	1	1	7	1	4	3	1	7	2	2	5	6	7	118
II	Accepted..	4	1	3	2		1															11	
	Rejected..				3	2	6	4	4	1	2	1	4	3	2	3	5	1	5	2	2	4	74
III	Accepted..	3	4	7	3																	21	
	Rejected..		4	1	2	4	1	2	2	4	3	6	1	3	2	2	2	1	1	3	1	2	60
IV	Accepted..	3	1	2																		11	
	Rejected..				1					3	2	6	4	1	1	3	2	6	4	5	3	2	57
V	Accepted..	5	6	1	3																	15	
	Rejected..		1	4	3	2	3	3	2	2	1	2	2	1	1	3	2	2	1	1	2	1	50

April 5: CULTURE D
Starved 24 hours[illegible]

These results from individuals are therefore strictly comparable and in accord with those obtained when a large number are tested at one time (table 1).

Now, in order to explain the cause of the change in reaction the suggestion might be offered that *Bursaria* shows a decrease in the rate of feeding because of the decrease in the amount of space in the body which food can occupy. This is undoubtedly true to some extent in those individuals which do not stop feeding until the cell becomes distorted by the comparatively immense mass of food. So far as the volume capacity of a normal individual of *Bursaria* is concerned, hundreds of observations have shown me beyond doubt that this may frequently be as much as *twenty-five to thirty grains*. Nevertheless, reversal of the cilia always takes place sooner or later. But the suggestion evidently does not apply to those individuals which show a change in the reaction when only a few grains have been eaten, for it seems impossible to understand how there could be a difference of as much as twenty grains of fresh yolk (table 2) in two normal individuals of equal size, when the cells are under exactly the same conditions, if this result were not due to a difference in the physiological state of the cells. Change in feeding was caused by the periodic reversal of the cilia and the reversal of the cilia in turn in some manner initiated or caused by a stimulus from the food already eaten, for it seems most natural to suppose that the stimulus originated from the change produced by the food mass in the interior of the cytoplasm. The most definite evidence that the change is due to stimulus from the eaten food is found in the radical change in the action of the cilia of the feeding mechanism.

If such fed individuals as those in table 3 are left in tap water free from food they may again eat yolk after digestion is partially or wholly completed, and again show a similar decrease in the rate of feeding, that is, a reversal of the oral cilia. The total quantity which will be eaten may be greater than that eaten at the previous feeding; but it usually is less, or often none at all.

.

The process of feeding in *Bursaria* shows it to be a functionally equilibrating system in its behavior towards food and the condition of its equilibrium at any particular time constitutes the physiological state which the cell is in, so far as its relation to food is concerned. The changes in the increase or decrease in the quantity of food eaten in successive meals and the increase or decrease in the rate of feeding might be discussed in the psychological terms 'hunger' and 'satiation,' but it is evident that the simpler terms quantity and rate express the facts of experiment, while any attempt at definitely determining whether the changes in quantity and rate are the same or different from 'hunger' and 'satiation' will obviously lead nowhere. Hence it seems better to use the terms, rate and quantity, which have a clear and quantitative meaning.

3. Other causes of individual variation

Bursaria at times closes up its oral apparatus. This may take place to such an extent that the opening is smaller than the food particles and then the latter can of course not be eaten. This condition can readily be observed under the binocular and it can always be determined beforehand whether closure has taken place to such an extent that the organisms can not feed. Other minor accidental individual variations are also present to some extent. These may be partly due to the difference in the size of the grains of yolk eaten. Sometimes when an individual is weak, owing to prolonged starving or for some other reason, two or three grains may become stuck in the oral pouch and this prevents feeding until the animal succeeds in throwing them out or by other means they become loosened. The material used was always examined beforehand to make sure that it was in a healthy condition so that these accidental conditions play no part in the final results of the experiments described.

Such a series of experiments as the foregoing do not show us specifically what these complex conditions are which have been cloaked in the phrase 'physiological states.' This however is

not the object of the above experiments: they are only here considered for the purpose of demonstrating the existence of these conditions, the fact of change within them and especially in this connection their rôle in the *external* phenomena of feeding and food selection in *Bursaria*, and how they may affect the results which will be given in the following pages.

EXTERNAL RELATIONS OF THE FEEDING PROCESS

1. Effects of external factors on feeding

a. Concentration of the food supply. The rate of feeding is within comparatively wide limits not dependant upon the concentration of the yolk suspension, provided it is not too low. This may be illustrated from one out of a series of experiments. The time of feeding was reduced to five minutes for the purpose of bringing out the effect of difference in the concentration more strongly. If the animals had been left in the suspensions twenty minutes (the usual time of feeding; cf. table 3) the difference would have been less marked, especially with material which shows a high rate of feeding.

*Experiment I.*⁵ Material from a healthy culture was starved twenty-four hours in tap water. All were perfectly normal and active. The experiment was carried out in 8 cc. stender dishes. The concentration in dish *B* was 8 times that in dish *A*. Twenty individuals were placed in each dish. The results from trial number 2 represent more nearly the ideal because these two suspensions were kept uniformly distributed during the five minutes feeding, and the individuals were picked out alternately by fives. Both trials, however, express equally well the proportional effect of concentration, namely, 1 to 2, as compared to the proportion of concentration, 1 to 8⁶ (table 4).

The concentrations used in the experiment are approximately represented by figure 3.

⁵ The experiments given in this paper are numbered in regular order for the convenience of the reader, and do not represent the actual order. Only a small number of the experiments actually carried out are given.

⁶ In all the experiments considered in this paper, where the concentration plays a part, the concentration was intermediate between those used in this experiment (fig. 3).

TABLE 4
Experiment I

TRIAL	DISH	NUMBER OF YOLK GRAINS IN EACH INDIVIDUAL																				TOTAL	AVG. PER IND.	
																							grains	
1	{	A	1	2	1	1	5	5	1	2	1	2	1	0	0	0	1	0	3	2	3	1	32	1.6
		B	1	2	6	3	7	4	4	7	5	1	2	0	2	3	1	4	3	3	1	3	62	3.1
SAME YOLK SUSPENSIONS USED																								
2	{	A	2	4	2	1	1	2	1	5	5	3	0	1	4	0	4	3	0	4	1	2	45	2.25
		B	1	8	6	1	4	4	3	8	4	1	5	7	6	7	2	4	1	0	7	2	81	4.05

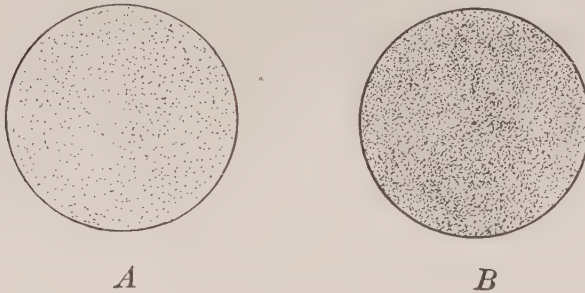


Fig. 3 Showing the relative concentration of yolk in dishes A and B of Experiment 1.

b. Effect of mechanical stimulation and of mechanical injury on feeding.

Experiment II. Thirty individuals from the same culture, starved twenty-four hours, were placed in each of six 8 cc. dishes containing 5 cc. tap water. Before feeding, the animals in three of the dishes (Set 1 in the experiment) were mechanically stimulated by means of a pipette. The opening of the latter was about ten times the width of Bursaria. The edges of the opening were made smooth by melting. The animals of Set 1 were stimulated by drawing them up into the pipette along with the tap water in the 8 cc. dishes, four times. Equal quantities of yolk suspension were now added to all the dishes. After having fed ten minutes the animals of Set 1 were again stimulated by drawing them along with the yolk suspension into the pipette two times; at the same time the control, Set 2, was stirred by gently shaking the dish and not allowing any instrument to touch the animals; hence the distribution of the yolk was the same in the two sets of dishes. All the individuals in Set 1, after having been stimulated, were perfectly normal and not injured. They looked like those of the

TABLE 5
Experiment II
SET 1. STIMULATED

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																											TOTAL	AVG. PER IND.			
																													grains			
A.....	0	1	4	0	1	0	1	3	1	5	1	1	2	0	1	1	0	1	1	1	0	2	2	0	1	3	0	0	0	33	1.1	
B.....	1	0	0	2	0	0	0	0	2	0	0	2	0	0	0	2	0	0	3	0	0	1	0	1	1	0	0	0	1	1	17	0.56
C.....	4	0	0	1	2	1	2	2	6	0	1	1	1	3	3	1	1	1	1	1	3	0	0	4	0	3	0	0	3	2	48	1.60
Total average.....																														1.08		

SET 2: CONTROL: NOT STIMULATED

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																								TOTAL	AVG. PER IND.						
																										<i>grains</i>						
A.....	3	5	7	2	3	6	3	3	3	4	14	13	3	1	2	5	8	2	9	5	9	5	10	3	7	7	6	3	4	2	157	5.23
B.....	2	1	3	6	3	1	5	1	3	6	4	4	5	2	3	2	5	6	11	4	2	6	6	1	3	2	7	2	3	0	109	3.63
C.....	6	1	3	6	5	6	5	3	2	7	4	4	4	3	3	6	5	4	3	2	6	1	3	2	3	5	6	2	2	1	113	3.76
Total average.....																												4.206				

control. If a smaller pipette is used or a larger one, and the stimulation, by sucking them along with the medium up into the pipette, is more violent, it will stimulate and injure the organisms so that they will not eat at all, or at least, not for some time after stimulation. Of course structural injuries are very easily produced, with the result that regulation of the cell must take place before any food can be eaten (table 5).

Proof that in this experiment, Set 1, if not in Set 1 of Experiment III, the organisms were not injured beyond the capacity for swallowing, is found in the fact that the great majority did eat, though only a comparatively small number of grains. Another experiment may be given to illustrate the same fact.

Experiment III. The animals in Set 1 were not stimulated before feeding, but after they had fed for five minutes they were stimulated by drawing the suspension with the animals in it, up into the pipette only once. Material from a different culture was used in this experiment; time of feeding fifteen minutes. The control suspension with the organisms was redistributed once by gently shaking the dish. The animals were all normal in form at the end of the experiment (table 6).

In Experiment III the stimulus was only slight as compared to that in Experiment II, yet the effect was marked. As stated above, strong stimulation may totally prevent feeding.

TABLE 6
Experiment III
SET 1: STIMULATED

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																												TOTAL	AVG. PER IND.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															

SET 2: CONTROL: NOT STIMULATED

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																												TOTAL	AVG. PER IND.		
																															<i>grains</i>	
A.....	6	9	8	6	6	4	10	7	6	5	9	7	7	1	7	2	5	9	9	14	6	7	6	7	6	0	10	7	9	6	201	6.70
B.....	6	2	4	6	6	1	7	9	5	4	6	4	6	2	7	1	6	10	0	4	7	2	5	5	5	6	7	10	6	3	152	5.06
C.....	0	11	4	6	6	9	8	6	6	9	6	5	5	9	7	7	9	2	7	4	7	8	8	7	12	6	8	6	9	1	198	6.60
Total average.....																													6.12			

The effect of inmechanical stimulation must be emphasized because it shows that in any work of this nature it is necessary to handle the organisms gently. This relation must be inferred to apply to work on other Infusoria also, at least to some extent.

c. Effect of temperature on feeding.

Experiment IV. Thirty individuals starved twenty-four hours, were placed in each of six vials. Each vial contained 5 cc. of tap water. These vials were now placed in large dishes containing water kept at the desired temperatures. The latter were read on a small thermometer set inside of each vial. Equal quantities of fresh yolk suspension were added when the temperature had reached the desired point. They were fed fifteen minutes (table 7).

TABLE 7
Experiment IV

		NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																								TOTAL																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
deg. C.		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0</

The experiment was repeated with closely similar results. At lower temperatures the animals are always unable to eat. As the temperature is raised and the activity of the cell increases, the rate of feeding increases, continuing to increase nearly up to the point where the cell is injured or killed by the heat. At temperatures between 20° and 25°C. (i.e., at about the optimum) the increase in the rate of feeding can be determined only by using a very large number of individuals, since the variations obliterate the effects when a small number is used.

All the experiments relating to other conditions were carried on at temperatures ranging between 20° and 27°C. Where necessary (as in prolonged experiments on digestion) the temperature was kept constant to within 1° to 1.5°C., throughout the course of the experiment, by keeping the organisms in moist chambers in a constant temperature oven.

d. Effect of HCl and NaOH on the feeding reaction.

Experiment V. The medium used in this experiment (table 8) was conductivity water.⁷ Any water less carefully purified is worthless for such experiments, as was shown by experiments carried out with tap water. By comparing the results it was strikingly evident that the acid and base had reacted with the salts and other impurities in the tap water and hence their effect was removed in low concentrations. The animals were washed once in conductivity water before putting them into the solutions. Time of feeding, twenty minutes (table 8).

It is seen from table 8 that the base NaOH was much more toxic than the HCl, and that as the concentration was increased the number of grains eaten became less and less. The chemical relations of the food and medium will be considered in more detail, later on (p. 29).

e. Effect of strong white light on the feeding reaction. Bursaria, when kept in dishes with a rather clear medium, often collect in the greatest number on the side of the dish away from fairly strong white light. It therefore became of interest to test what effect continuous light of a high intensity would have upon the rate of feeding.

⁷ Prepared and used in the Department of Physical Chemistry for conductivity measurements.

TABLE 8
Experiment V

NaOH

MOL. CONC.	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																TOTAL NUMBER GRAINS				
1/400.....	All dead in 3 minutes																0				
1/600.....	All dead in 10 minutes																0				
1/800.....	Many dead at 15 minutes; none eaten at end of 20 minutes.....																0				
1/1200.....	All alive and normal in shape at end of ex- periment; no grains of yolk eaten																0				
1/1600.....	0	1	0	0	0	1	1	0	2	1	2	2	0	1	3	2	0	3	0	0	19
1/3200.....	6	8	8	7	3	5	1	3	1	9	5	5	10	9	6	11	4	7	5	8	121
1/6400.....	5	9	8	8	6	14	8	1	11	5	8	5	12	4	8	11	6	8	6	5	148
CONTROL IN CONDUCTIVITY WATER																					
	7	9	10	9	9	4	5	10	10	6	6	12	4	6	5	6	17	8	8	4	155

H Cl

1/400.....	All dead in 5 minutes.....																				0
1/600.....	0	1	2	1	1	1	2	0	2	1	4	2	0	1	2	0	5	4	1	2	32
1/800.....	1	3	4	5	13	4	5	5	2	6	0	5	4	4	3	3	7	5	5	6	90
1/1200.....	6	8	1	8	7	8	6	0	6	3	8	3	1	5	4	1	1	2	11	1	90
1/1600.....	6	5	8	8	7	9	3	12	3	2	5	8	14	6	9	3	5	5	0	5	123
1/3200.....	8	7	15	5	11	13	8	9	0	2	4	1	3	8	2	10	5	5	6	8	130
1/6400.....	5	10	12	10	9	7	9	3	14	6	10	5	9	9	10	10	7	9	5	3	162
CONTROL IN CONDUCTIVITY WATER																					
	12	12	4	13	0	11	14	5	6	2	0	9	7	8	2	11	3	11	3	4	137

Experiments VI and VII. White light from the arc of an Edinger apparatus was focused upon the stage so that a spot of light $1\frac{1}{2}$ inches in diameter, of a very high intensity, was obtained. The light was filtered through a layer of water 1.5 cm. in thickness. An 8 cc. stender dish containing thirty normal individuals was placed in the spot of light and the usual quantity of yolk suspension added. A control was kept in weak diffuse daylight. The animals were fed twenty minutes. The following results show that continuous action of intense white light on the animals does not have any effect upon the rate of feeding. Two experiments with controls are given (tables 9 and 10).

TABLE 9
Experiment VI

STRONG WHITE LIGHT

NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																				TOTAL
2	6	5	6	11	8	2	5	8	8	1	2	3	5	6	6	3	6	3	3	7
2	7	6	6	5	6	1	5	6												150

CONTROL: DIFFUSE DAYLIGHT

8	5	6	4	3	6	0	10	0	5	4	4	1	2	4	3	3	7	3	6	7
7	7	8	6	7	4	7	9	2	5											146

TABLE 10
Experiment VII

STRONG WHITE LIGHT

NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																				TOTAL
5	6	9	6	3	1	5	3	3	5	8	1	7	6	4	11	7	7	0	2	10
4	3	4	4	5	11	3	6	5												154

CONTROL: DIFFUSE DAYLIGHT

8	8	2	5	3	10	9	11	8	9	2	7	8	1	0	4	15	0	8	2	8
10	6	7	6	8	11	3	4	5												188

f. Effect of the electric current. Weak induction currents have, within a limited time, no noticeable effect upon the feeding, as is shown by the following results from two separate experiments, VIII and IX. The total number of grains eaten by 20 individuals in each of two 8 cc. dishes is given in each experiment:⁸

Experiment VIII

Dish A—73, total number of grains eaten by 20 individuals

Dish B—69, total number of grains eaten by 20 individuals

Experiment IX

Dish A—65, total number of grains eaten by 20 individuals

Dish B—64, total number of grains eaten by 20 individuals

Control for Experiments VIII and IX; not stimulated by the current

Dish C—89, total number of grains eaten by 20 individuals

⁸ The apparatus was arranged in such a way as completely to prevent any effect of substances liberated at the electrodes, by inserting the electrodes in a physiological normal NaCl solution in each of two 8 cc. dishes and from these the circuit was closed through the other two 8 cc. dishes containing the animals, by means, of small n tube connections filled with tap water and plugged loosely with a wad of cotton.

When, however, a direct current is used of such strength that the organisms can be made to go to one side or the other by reversal of the current the effect becomes more or less apparent. Feeding can not be prolonged to twenty minutes with a strong direct current, for the organisms are easily injured. To obviate this, time of feeding was limited to five minutes. The animals were made to swim from one side to the other by frequent reversals of the current. In Experiment X, they were stimulated by frequent reversal of the direct current during the first minute of feeding and then left to feed four minutes more without stimulation. In Experiment XI they were stimulated in the same way during the whole period of feeding. Time of feeding, five minutes (tables 11 and 12).

TABLE 11
Experiment X

STIMULATED BY DIRECT CURRENT, 1 MINUTE

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																TOTAL
																	grains
A.....	3	13	5	11	3	10	8	0	1	6	11	5	8	4	3	7	111

CONTROL: NO CURRENT

B.....	7	6	4	6	3	8	10	7	7	6	8	5	0	4	3	3	9	4	10	8	118
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TABLE 12
Experiment XI

STIMULATED BY DIRECT CURRENT, 5 MINUTES

DISH	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																TOTAL				
																			grains		
A.....	1	3	4	6	2	3	10	6	5	10	5	0	0	2	3	8	7	1	3	11	90

CONTROL: NO CURRENT

B.....	5	5	11	0	2	18	3	11	1	11	0	11	13	10	0	5	7	0	9	5	6	122
--------	---	---	----	---	---	----	---	----	---	----	---	----	----	----	---	---	---	---	---	---	---	-----

As the results show, feeding was not discontinued under these conditions of strong stimulation by the current, though the or-

ganisms show a somewhat smaller total number of grains eaten than in the controls, in the same length of time. The difference is, however, too small to have a clear significance.

The strength of current may be increased but usually feeding can never be totally inhibited unless the organisms are injured or killed immediately after the yolk has been added.

The preceding experiments show clearly the relation which exists within certain limits, between the feeding reaction of this organism and a simultaneous reaction to certain other types of stimulation. During stimulation with HCl and NaOH, and especially with high temperatures and the electric current, the notable fact is that the reaction to food is strongly persistent under wide ranges of intensity of a second applied stimulus; this is true to such an extent that under some conditions the feeding continues up to the point where the intensity is so high that the stimulus is destructive to the organism. These facts must not be thought to be of general application, for evidently mechanical stimulation is quite effective in changing the reaction to food. What the behavior will be under two simultaneous stimuli obviously depends upon the nature of those stimuli.

It should be distinctly noted that in all the foregoing experiments the chemical as well as physical nature of the food substance has been kept constant while the organism in its particular physiological state has been acted upon by certain external agents; these being of a sufficient variety to indicate clearly what rôle these different types of factors play in the relation of this animal to food, and to serve as a guide to further inquiry.

We now have to see what changes are produced in the feeding reaction by modifying that factor which in the foregoing experiments has been kept constant,⁹ namely, the food. In the following series of experiments all the other conditions will be kept constant, or at least arranged in such a way that they may be

⁹ An exception to this might be taken in the experiments with HCl and NaOH for it is a question whether or not these affect the chemical character of the yolk sufficiently under the conditions of these experiments to modify the number of grains eaten. The yolk was not treated previous to the feeding; thus the time was so short and the dilutions so high that any change must have been very slight.

properly controlled and accounted for. We shall attempt to determine what the relation of Bursaria is, to specific physical and chemical properties of the food itself. First it will be determined how the external part of the reaction is modified, that is, what is the behavior of the cell in so far as this has to do with the selection of food.

SELECTION OF FOOD AND THE FACTORS CONCERNED

The object of the experiments described in the present section is to answer the question: Can Bursaria discriminate quantitative or qualitative differences between the yolk grains?

When fresh hard boiled yolk grains, prepared as described on page 8, are treated with different kinds of water-soluble dyes, the amount of dye which is adsorbed by a grain of yolk varies with the kind of dye used. At first a considerable number of different dyes in aqueous solution were tested in a comparatively rough way; first, for the relative amount of each dye which would be taken up by the grains of yolk; second, for the rate at which the dyes were adsorbed and the ease with which they could be washed out (reversibility of the adsorption); and third, for the relative toxicity of aqueous solutions of these dyes to the organisms. Among the dyes so tested were fuchsin, lyons blue, methylin blue, eosin, cyanin, gentian violet, saffranin, janus green, congo red, and an aqueous solution of hematoxylin.

The results of the following experiments on food selection, in so far as they are related to the dye, depend upon the three factors named: (1) The amount of dye adsorbed (2) The rate of the reversible adsorption reaction, and (3) The relative toxicity to Bursaria, of the dye in aqueous solution.

It was quickly found that certain dyes were better suited than others, for the particular end in view. Aqueous solutions of saffranin and janus green were found best to fulfil the necessary conditions. Both show a reversible adsorption with yolk, while the velocity of the reversible adsorption is sufficiently low to prevent a too rapid washing out of the stain. By this means one is able to control the amount of adsorbed dye much more

easily than if it could be washed out quickly, and one is also able to control the concentration gradient between pure water and the dye adsorbed by the yolk grain. The toxicity of the different dyes varies greatly, and it was found that saffranin and janus green were best from this point of view also, since both of these are very toxic to *Bursaria* in higher concentrations but only slightly so in lower concentrations.

1. Experiments with stained and unstained yolk

a. Saffranin.

Experiment XII (a). Object, to test (a) whether or not *Bursaria* will eat yolk grains which have adsorbed an appreciable amount of the soluble toxic substance saffranin and (b) whether or not the amount of yolk eaten depends, in this experiment, upon the amount of saffranin adsorbed.

Equal volumes of a strong suspension of fresh yolk were placed in each of seven stender dishes of 8 cc. capacity. A bright rose-colored solution of saffranin was made up with tap water. To the dishes designated *A, B, C, D, E* and *F* was added 5, 4, 3, 2, 1, and 0.5 cc. respectively, of this solution, and mixed thoroughly. The seventh dish without stain, was kept as a control. The suspensions were left to settle five minutes, then decanted and 5 cc. tap water added to all the dishes; this was repeated three times. The organisms used were starved twenty-four hours and were in excellent condition. The time of feeding was fifteen minutes (table 13)

TABLE 13
Experiments XII (a)

DISH	SAFFRANIN SOL. C. C.	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																												TOTAL		
																														grains		
A.....	5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	5
B.....	4	0	0	0	0	4	8	0	3	1	0	0	0	0	1	3	0	1	0	0	0	4	0	3	4	0	1	0	0	1	0	34
C.....	3	3	0	6	0	0	2	0	8	3	1	2	8	4	2	4	0	1	0	0	2	3	1	1	0	2	1	2	4	0	1	61
D.....	2	2	0	0	3	1	1	0	2	4	2	1	2	2	1	3	3	2	0	6	5	5	0	4	0	0	0	0	0	8	4	56
E.....	1	4	3	1	2	7	6	2	1	7	9	0	3	7	2	0	12	6	4	0	4	4	5	4	1	6	5	0	0	7	1	113
F.....	$\frac{1}{2}$	4	6	11	6	1	2	4	0	3	0	3	9	3	4	3	6	5	4	5	9	9	6	5	3	1	3	8	2	7	6	138
Control																																
G.....	0	0	5	6	6	7	4	9	12	4	9	9	1	14	4	2	7	7	9	6	12	1	9	11	0	6	3	8	4	6	7	188

When stronger solutions of saffranin than that in *A* were used, no grains were eaten. All the animals at the end of the experiment were normal and had not been injured. The yolk of dish

A was now left to soak in its water for fifteen minutes longer, this water was then drawn off and the yolk again washed twice with water. Thirty individuals from the same material as used above were now put into the dish. At the end of fifteen minutes the following was the count:

Experiment XII (b)

3, 1, 5, 1, 3, 4, 4, 3, 9, 3, 7, 3, 0, 4, 5, 3, 6, 0, 0, 4, 1, 4, 5, 0, 0, 2, 3, 1, 3, 1. Total 88 grs.

This indicates that we may obtain the same result whether we proceed with a strongly stained yolk and test successively after each washing, or, as in the former experiment, by staining different portions of yolk to different degrees to begin with. This was actually done in other experiments not given, and results exactly similar to those in Experiment XII (a) were obtained.

To show that even a considerably stronger medium does not injure the animals seriously, a yolk suspension stained with saffranin more strongly than that used in A of Experiment XII (a), was made by leaving the yolk several hours in a very strong solution of the stain. This was washed out several times and then thirty individuals from the same culture material used in the former experiments were put into it and left for fifteen minutes. They were then picked out and washed once in tap water, and then transferred to an unstained yolk suspension for fifteen minutes. The count gave the following (table 14):

TABLE 14
Experiment XIII

MIXTURE	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																												TOTAL		
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
Unstained.....	0	2	0	3	0	0	0	0	0	1	0	3	1	0	0	2	1	3	2	2	3	2	8	0	0	0	0	0	2	0	35

This shows that they were not injured sufficiently in even this strongly stained suspension totally to prevent them from eating unstained yolk immediately afterward. The cause of most of the 0's in the count is that, as the toxicity of the solution increases, the organisms have a tendency to close up the oral apparatus and do not open it again sufficiently, within the next twenty minutes or so, to be able to take in the yolk grains. Of

course if yolk which has been very strongly stained and not washed out sufficiently, is fed, then the concentration of the medium rises so quickly that they are greatly injured or killed.

Now against the conclusions which will be drawn from the results of Experiment XII (a) and (b), as it stands, may still be urged the objection that the reason that so few or no grains are eaten, is because of what one might call a general injury or stimulation of the cell by the saffranin which is rapidly being liberated into the water, and that it may not have anything to do with a specific reaction to the chemical character of the food particle as such, that is, to anything like a "sense of taste." That this objection does not apply to conditions like those in the above experiment (XII, a and b) where the amount of stain adsorbed even in dishes *A* and *B* is very little compared to that in Experiment XIII, may be shown by taking the solution of dish *A*, Experiment XII (a), and placing unstained yolk and *Bursariae* in it. The result of such an experiment is that the organisms fill up with fresh yolk, showing that the medium in weaker concentrations does not affect the eating process to any appreciable extent. Further proof of this will be given in the experiments immediately following, and also in experiments to be given later.

Experiment XIV. To test whether or not *Bursaria* can select and eat non-toxic yolk grains from among toxic ones, when the two are mixed. Two suspensions were made, one of yolk stained in saffranin twenty-four hours, then washed out repeatedly, the other containing the same kind of yolk washed in the same way but not stained. The two yolk suspensions were mixed immediately before the animals were placed in the mixture. Twenty individuals were used. The time of feeding was fifteen minutes; a control of washed unstained yolk alone, was kept at the same time (table 15).

TABLE 15
Experiment XIV

MIXTURE	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																TOTAL
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	×	×	×	×	0
Unstained.....	0	5	2	3	2	1	2	4	2	0	1	2	3	2	×	×	29
Control:																	
Unstained yolk....	12	16	7	15	4	9	15	8	9	9	16	3	25	7	15	12	213

In the mixture the concentration of the saffranin rose so rapidly that some of the individuals were killed.¹⁰ This is indicated above by X. Yet even in such a strong solution, selection took place, though the number of grains eaten was small compared to the number in the control.

Experiment XV. Another sample of yolk less deeply stained than that in Experiment XIV, was washed out many times and mixed with an equal quantity of unstained yolk from the same sample. Thirty individuals of the same material as used in Experiment XIV, were fed for five minutes, instead of fifteen minutes as before (table 16).

TABLE 16
Experiment XV

MIXTURE	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																												TOTAL						
Stained.....	1	1	1	1	0	1	1	0	3	3	2	2	0	1	0	3	1	2	4	4	1	2	2	1	2	1	0	0	1	2	43				
Unstained.....	2	6	7	2	16	14	4	1	1	1	1	1	1	1	2	9	7	13	9	0	6	5	12	9	5	7	2	10	7	5	7	3	8	6	216

A repetition of the above experiment with yolk stained a little more deeply gave the following result; twenty individuals used; fed five minutes (table 17).

TABLE 17

MIXTURE	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																				TOTAL
Stained.....	0	0	3	1	2	2	1	0	1	1	1	2	1	3	0	1	3	2	1	0	25
Unstained.....	4	0	0	1	4	5	5	0	4	2	3	5	3	0	1	3	0	12	1	3	56

The control of Experiment XIV will likewise serve for Experiment XV.

The results of this experiment are to be explained by the fact that the concentration gradient of the adsorbed toxic saffranin is relatively low with respect to the gradient of the water-soluble yolk substance to which *Bursaria* reacts in a strongly positive manner. Of course one is not to suppose that it is the relative molecular concentration gradient alone that determines the re-

¹⁰ The cytolytic action of saffranin is in some ways more marked than that of janus green. The character of its reversible adsorption reaction also makes it less suited for use in experiments of this kind than janus green, as will appear from results with the latter.

sult. What is meant in this case by concentration gradient, is the molecular concentration plus the specificity of the substance, that is, in anthropomorphic terms we should say "the kind of taste" which the substance has. That the specific nature of the substance is one factor in determining the result, is shown by a comparison of the results of numerous experiments with saffranin, janus green, hematoxylin, and especially other less toxic stains, like congo red (cf. what follows).

b. Janus green. A considerable number of experiments have been carried out using this substance, with the same general results as those obtained with saffranin. It is better adapted to bring out the phenomenon of selection than saffranin, causing a sharp discrimination by *Bursaria*; small quantities adsorbed by the grains are sufficient to bring about rejection. The following experiments show some of the relations.

Experiment XVI (a) and (b). Yolk was stained in janus green twenty-four hours then soaked in tap water and washed repeatedly. A portion of the same kind of yolk soaked and washed in the same way but not stained, was used as a control and for mixing with the stained yolk. A few minutes before the experiment equal quantities of the stained and unstained yolk suspension were mixed in dish A. A second quantity of the unstained yolk suspension of a concentration equal to the sum of those in dish A was placed in dish B. Twenty individuals were placed in each dish and left to feed twenty minutes (table 18 a).

TABLE 18 (a)
Experiment XVI (a)

MIXTURE, DISH A	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																			TOTAL
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unstained.....	5	3	1	6	2	4	1	4	5	1	5	1	4	4	1	0	0	0	0	0
Control, Dish B:																				
Unstained.....	9	12	21	8	20	12	15	19	13	17	14	13	15	12	19	17	14	7	8	3

The yolk in both dishes was now washed twice and the experiment repeated with control. Time of feeding fifteen minutes. The count is shown in table 18 b.

In (a) the solution had become sufficiently strong to affect five of the animals (X), so that they could not be recovered for

TABLE 18 (b)
Experiment XVI (b)

MIXTURE, DISH A	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																		TOTAL
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unstained.....	0	0	0	1	0	0	1	1	2	0	1	2	2	4	0	0	1	0	13
Control, Dish B:																			
Unstained.....	16	8	17	7	13	10	11	12	5	12	4	5	12	15	15	16	7	10	216

the count. The smaller number of grains eaten in (b) by those in dish A is due in part to the shorter time of feeding but more to the fact that the unstained yolk grains had by this time ad-sorbed some of the liberated janus green from the stained yolk grains (see Experiment XVIII, p. 36).

Experiment XVII (a) and (b). The results of this experiment are given to show that Bursariae from two different cultures may show different reactions in selection experiments. In part (a) material was used from one wild culture, while in part (b) material from a different one was used. Both were starved twenty-four hours before using them. All other conditions were alike. Time of feeding fifteen minutes (table 19).

TABLE 19
Experiment XVII

MIXTURE, DISH A	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																		TOTAL
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unstained.....	0	4	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	9
Control: dish B, Unstained.....	5	4	6	2	4	7	6	3	9	3	8	3	3	6	4	3	2	7	99
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unstained.....	3	4	0	2	10	3	3	2	7	6	1	6	6	0	8	2	1	0	70
Control: dish B, Unstained.....	2	4	13	9	5	7	9	2	4	10	6	15	9	13	6	11	9	1	148

In such experiments as these it was found that occasionally an individual had eaten a stained grain along with the unstained ones, but this happened very seldom in any of the experiments with janus green.

Experiment XVIII. The two dishes *A* and *B* in Experiment XVII (b) were left standing for two hours; then they were washed once and tested with the same material used in Experiment XVII (b), in order to show the effect of the adsorption of the liberated stain by the unstained grains mixed with the stained ones. Time of feeding fifteen minutes (table 20).

TABLE 20
Experiment XVIII

MIXTURE, DISH A	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																TOTAL
Stained.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unstained.....	0	2	0	7	2	0	1	4	0	1	0	0	0	2	0	2	0
Control: dish B, Unstained.....	18	23	8	15	13	7	0	9	12	15	4	17	16	12	15	15	11
																0	8
																10	
																	228

This shows that when the janus green yolk is left with the unstained yolk for some time, the liberated stain from the janus green yolk is adsorbed by the unstained yolk grains, and as a result the latter are not eaten so readily. If the mixture is left standing too long and then rinsed in tap water, then no grains are eaten. *Bursaria* can react to such small quantities of adsorbed janus green that the amount adsorbed cannot be distinguished by the eye, when the unstained yolk grains mixed with the stained ones are examined.

Experiment XIX. To prove that the solution of the janus green which is produced by the liberation of the stain from the stained yolk, does not even in quite strong concentrations prevent the eating of fresh yolk placed in it, the result of one test out of a considerable number made at different times, is given. A solution was drawn off from janus green stained yolk used in an experiment in which *no* grains of the mixture had been eaten, after standing for some time. To this solution was added unstained yolk. Ten individuals were tested (table 21).

TABLE 21
Experiment XIX

	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL										TOTAL
Fresh yolk in janus green solution, dish A.....	8	3	6	3	1	0	3	0	3	0	27
Control, fresh yolk in tap water, dish B.....	5	15	4	5	0	6	5	3	1	0	44

It is evident that the solution of janus green drawn from the mixed suspension, and produced by the liberation of the stain from the stained yolk grains of the mixture which was not eaten, did not now prevent the animals from eating unstained yolk grains which were placed in it; hence it was not the stain in solution which prevented the eating of the stained grains of the mixture from which the solution was drawn; but the stain which was adsorbed by the yolk grains of the mixture and diffused from them. Many such similar tests were carried out giving the same result. This does not mean that the solution apart from the yolk grain with its adsorbed dye, may not affect the result of the feeding, for in higher concentrations the solution apart from the stain upon the grain does affect the feeding process. In solutions of lower concentrations of the appropriate dye the chemical nature of the grain along with the amount of dye adsorbed, are the essential factors determining the number of grains which will be eaten.

c. Hematoxylin. To show further that the specificity of the toxic agent plays a large part in determining whether or not yolk will be eaten, the following experiments are given. It will be noted that in this case we have a substance which has a very different effect upon the cell and its relation to food, from that produced by the substances thus far dealt with. The solution in this case may be made very deep brown while the grains are also stained deeply, and yet the yolk grains are eaten even in solutions which kill the animals if they remain in it more than three or four minutes.

Experiment XX: Table 22 (a) and (b). The same quantity of yolk was added to each of nine dishes of 8 cc. capacity, each containing equal amounts of tap water. The dishes were numbered 1, 2, 3, and so forth. To these were added diverse quantities of the 0.5 per cent aqueous solution of hematoxylin by drops, as given in the tables; time of feeding ten minutes.

This experiment was repeated with the same suspensions at the end of one hour; time of feeding fifteen minutes (table 22 b). The individuals which died before the count was made are denoted by X. The tables show that although the solutions, espe-

cially in higher concentration, are very injurious, the organisms, nevertheless, eat the grains of yolk. After one or more hours the grains become stained deeply. This was the case in table 22 (b). The increase in the length of time of feeding (i.e., the time the animals were left in the solution) is the cause of the high mortality in table 22 (b).

TABLE 22 (a)
Experiment XX

NUMBER OF DISH	1	2	3	4	5	6	7	8	9
Number of drops of $\frac{1}{2}$ per cent aq. hematoxylin.....	4	8	12	16	20	24	28	50	0
	0	0	0	2		1	4	7	7
	4	1	2	3		4	6	0	6
	6	2	0	11		7	3	×	10
Number of grains eaten by each individual.....	2	5	4	1		3	7	×	11
	2	4	0	1	lost	1	1	×	13
	0	0	0	1		11	1	×	8
	4	1	1	2		7	4	×	14
	6	7	3	4		0	4	×	6
	0	3	0	13		2	2	×	6
	4	0	7	×		4	×	×	8
Total.....	28	23	17	38+		40	32+	(7+)	89

TABLE 22 (b)

NUMBER OF DISH	1	2	3	4	5	6	7	8	9
Number of drops of $\frac{1}{2}$ per cent aq. hematoxylin.....	4	8	12	16	20	24	28	50	0
	3	1	0	1	1	0	×	×	11
	5	1	1	2	×	3	×	×	8
	0	2	0	1	×	0	×	×	7
Number of grains eaten by each individual.....	3	3	4	0	×	2	×	×	11
	2	0	3	0	×	×	×	×	14
	5	6	0	0	×	×	×	×	8
	4	0	0	×	×	×	×	×	6
	5	3	0	×	×	×	×	×	8
	3	1	0	×	×	×	×	×	13
	3	×	×	×	×	×	×	×	7
Total.....	33	17+	8+	4+	1+	5+	—	—	93

That the yolk grains are eaten, though to a less extent, even after having been left in the solution of 50 drop concentration for three and one-half hours, is shown by the following: Feeding was limited to three minutes, which in part accounts for the comparatively small number of grains eaten. Ten individuals were used. The count gave 4, 2, 1, 6, 1, 1, 2, 0, 0, 1, a total of 18 grains of the deeply stained yolk. When this deeply stained yolk in the 50 drop concentration of hematoxylin was washed four times in tap water and tested again; the following count was made: 11, 13, 14, 6, 5, 2, 12, 2, 4, 5, a total of 74 grains.

These tests show (a) that although a dye may be toxic to Bursaria, it may nevertheless not affect, to any great extent, the functioning of the feeding mechanism in the taking in and swallowing of the food, though (b) with some dyes total rejection of the food may take place, when the concentration is so low that it has only a comparatively slight cytolytic effect. The former condition is shown to a less marked extent in the experiments with saffranin than in the experiments with hematoxylin; while the latter condition is illustrated by the results with janus green. This seems then also to strongly suggest that different substances may affect different parts of the cell differently. Corroborative evidence upon this point, which it would be out of place to consider here, has been obtained from observations showing that the localization of the beginnings of cytolysis of the cell body of Bursaria may differ with the particular nature of the toxic agent employed.

d. Congo red. Another stain which is adsorbed readily is congo red. This, however, unlike hematoxylin, can only be washed out in part, that is, its adsorption reaction is not completely reversible. Also since this dye is not as toxic as saffranin or janus green, a large quantity of the stain may be adsorbed and yet not appreciably affect the number of grains eaten, as is shown by the following experiment.

Experiment XXI. The yolk was stained twenty minutes in a strong aqueous solution of the dye. Time of feeding twenty minutes. Thirty were used (table 23).

TABLE 23
Experiment XXI

NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																											TOTAL				
Congo red, dish A																															
Stained.....	7	3	6	1	7	6	2	5	1	2	5	4	4	3	3	2	4	4	4	0	5	1	7	3	2	4	6	5	5	4	115
Control dish B																															
Unstained.....	11	8	7	3	6	8	5	6	6	5	8	7	5	1	2	5	6	7	1	5	8	6	7	10	5	6	5	9	4	7	179

In this case we have a comparatively low concentration gradient of the dye, together with a low toxicity and hence the comparatively small difference in the readiness with which *Bursaria* eats the stained and unstained yolk.

e. Sudan III. To show that an adsorbed substance which is insoluble in the medium has no determining effect upon the feeding and food selection, Sudan III was employed. This substance is insoluble in water but soluble in ethyl alcohol and fats.

Experiment XXII. Fresh yolk was stained in an 80 per cent alcoholic solution of Sudan III for a short time. It was then dried in an oven at 27°C. for twenty-four hours. A control of fresh yolk was also kept. The organisms were fed twenty minutes. The yolk takes on a very deep color with this stain.

TABLE 24
Experiment XXII

NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																					TOTAL
Sudan III, stained																					
yolk.....	13	18	19	0	15	3	5	7	18	25	15	5	7	8	9	17	18	12	3	26	243
Control, unstained..	18	3	4	14	7	15	17	22	6	28	20	6	9	3	29	18	13	10	8	24	274

It is evident that the insoluble Sudan III had no appreciable effect upon the food reaction. Mixtures of these showed no difference in the amounts of the two kinds of yolk eaten.

f. Stale yolk.

Experiment XXIII. Mixtures of fresh and stale yolk could not be used since the grains of the two kinds of yolk were visibly indistinguishable. One experiment is given. The stale yolk was four weeks old while the control was freshly prepared; both were of the same concentration (table 25).

TABLE 25

Experiment XXIII

	NUMBER OF GRAINS EATEN BY EACH INDIVIDUAL																			TOTAL	
Stale yolk.....	0	2	5	1	0	3	8	2	1	1	4	0	6	1	5	1	3	1	1	3	48
Control, fresh yolk.	8	4	7	2	1	1	4	4	9	3	0	7	9	5	2	2	6	1	0	1	96

A difference is plainly evident. Other experiments show greater or less difference, depending upon the conditions.

2. The basis for and nature of food selection in Bursaria, as shown by the foregoing and other experiments

It must be remembered that in any such experiments as the foregoing the relation to food is in some ways an entirely new one to the organism. Yet it must be insisted upon that the yolk used in these experiments is assimilable by the organisms (a fact which will be considered at length in a later paper) and especially that whatever the mechanism of feeding and selection in nature is, it must be the same one which is brought into action in these experiments. Hence the criticism imagined above would appear to have no importance for the question under consideration here. In fact, it is to be believed that so far as these experiments are concerned, they are only a more strongly emphasized condition of what we find in nature and that they picture to us, so far as they go, the actual condition of the food relation of Bursaria in its native culture.

We may state the results briefly in the following way: First: Yolk grains are rejected if the soluble adsorbed toxic substance makes with the medium a sufficiently steep concentration gradient. If this gradient is low relative to that of the yolk-soluble substance, to which Bursaria reacts positively, then the organism may eat the stained yolk, other conditions being equal. Second: (a) Whether Bursaria will eat stained yolk grains or reject them depends also, along with the steepness of the concentration gradient, upon the specific chemical properties of the adsorbed sub-

stance in question, and furthermore (b) the substance by virtue of its chemical properties has, at least in some cases, a specific action upon the mechanism of feeding and selection, as is shown by a comparison of the results of the experiments with hematoxylin, saffranin and janus green. Additional evidence obtained from observations upon the phenomena of cytolysis in *Bursaria* also points to the correctness of this conclusion. A familiar instance of a similar nature is the casting off of the peristome by *Stentor* when stimulated or injured by chemicals. Another instance is the fact found by Jennings that the anterior end in *Paramecium* is more sensitive to mechanical stimulation than are other parts of the body.

That in feeding experiments with the Protozoa it is difficult to discriminate closely between the effects of the medium and those of the food substance itself is obvious, since (a) the amount eaten depends upon so many factors other than the nature of food and (b), since the organism selects on a chemical basis, which involves a soluble substance or substances diffusing into the medium from the food particle, hence necessarily involving the external medium to a greater or less extent. It is of course clear that differences in certain physical characters of food may likewise determine whether or not it will be eaten. This is shown most simply by objects which are too large, such as large yolk grains and large individuals of *Stentor*, which cannot be swallowed.

From all the facts found from experiments upon food selection by *Bursaria*, there is no evidence that active selection is based upon either "size, weight, form or surface texture" or any combination of these, except in so far as simple mechanical conditions would make them effective. All the facts show clearly that the chemical nature of the food is the property upon which the power of discrimination by *Bursaria* depends. Hence I find no evidence from *Bursaria* to support Schaeffer's contention that "*Stentor* selects its food upon a tactual basis and apparently not upon a chemical one" and that "*Stentor* reacts in selecting food, to physical properties only or chiefly, and not to chemical properties" (Schaeffer '10, page 131). On the other hand, the

facts which have been found in this connection are in agreement with the results and conclusions so far as they have been worked out by Metalnikow ('12) for *Paramecium*.

THE RELATION OF BURSARIA TO DIGESTIBLE AND NON-DIGESTIBLE SUBSTANCES

1. The external relations

Many substances which are in the ordinary sense chemically indifferent to the organism are likewise eaten, though generally in small quantities. Among these are cinnabar, carbon black, chinese ink, powdered aluminium and the like. The relation of *Bursaria* to this class of substances is however strikingly different inside of the cell and to a large extent outside, when compared to that relation in the case of digestible and assimilable ones. The fact that some comparatively indifferent substances like the above, are eaten does not affect our conclusion drawn above, as to the paramount importance of the chemical properties of the food in food selection. Chinese ink contains some mucilaginous matter which as my own observations have shown me, is reacted to positively by *Bursaria* and hence the ink is quite readily eaten. Carmine is a similar substance which though generally taken to be insoluble in water, is in fact sufficiently soluble clearly to affect the feeding reactions of *Bursaria*. Furthermore, the fact that a substance may be insoluble does not, of course, prove that the stimulus from it is not a chemical one, for it is probable, that with such substances as aluminium, catalytic or other specific chemical, or even physical reactions dependent upon the chemical properties of the substance, are produced by contact with the plasma membranes. The possible variety of interactions of the cell with different kinds of substances when considered in this order of magnitude may of course be very large.

As regards the eating of non-digestible substances, powdered aluminium may serve, in one way, to illustrate the external relations. If a large number of individuals are put into a suspension of aluminium, often few if any will eat any of the particles of

aluminium and those that do eat it generally take in only a small quantity. This is also true of Sudan III and of carbon black. The quantity eaten varies with the conditions in a similar way, as previously set forth for yolk. Now if fresh yolk grains are added to the suspension of aluminium the animals will often quickly fill up with yolk, but in this case flakes of aluminium become attached to the yolk particles and hence often considerable quantities of the metallic aluminium are passed into the body along with the yolk. Sometimes the quantity of yolk eaten in such a mixed suspension is less than that in the control. This serves to illustrate the sort of equilibrium which exists between the organism and the kinds of substances in suspension, partly determining the amount of food and other substances eaten.

2. The internal relations

It was interesting to find that *Bursaria* possesses what I shall call an internal compensating reaction to those substances which are eaten to some extent, but are not digestible, such as Sudan III, chinese ink, powdered aluminium, and so forth. This compensating reaction makes up to some extent, in the "economy of the organism" for the lack of a perfect discrimination between indigestible ('tasteless') substances and those which can serve as food. It is shown by the fact that indigestible substances are eliminated from the cell usually a long time before the digestion of a similar quantity of food is completed. This may be shown with Sudan III. The results of the experiments are, for the sake of brevity, given by curves.

Experiment XXIV: Figure 4. Three sets of twenty-four individuals each were fed Sudan III, cold-ether-extracted yolk, and fresh yolk respectively. They were placed two in each watchglass containing tap water in moist chambers, and examination in this case was made at the end of three, seven, and twenty-two hours. Points on the abscissae indicate the length of time in hours after feeding, while points on the ordinates show the number of individuals which had extruded Sudan III (curve *A*) in the time intervals between the examinations, or in the case of extracted yolk (curve *B*) and fresh yolk (curve *C*) the number of individuals that had lost all traces of food. In this experiment the observations were not sufficiently frequent to bring out the actual

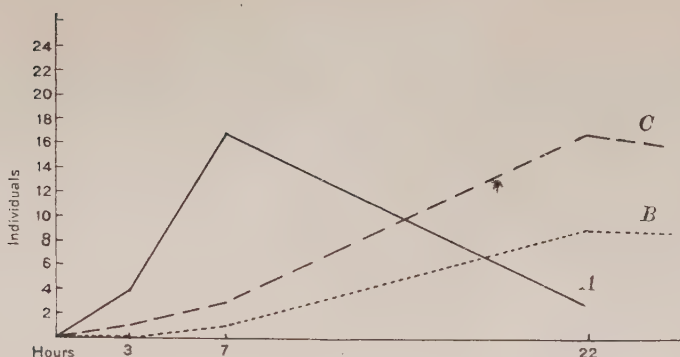


Fig. 4 Experiment XXIV. Curve A represents the course of total extrusion of Sudan III; curve B, that of complete digestion of cold-ether extracted yolk; curve C, course of complete disappearance of fresh fat-containing yolk.

course of the extrusion of Sudan III or of the disappearance of the yolk from the cytoplasm, but it will be noted that the great difference appears in the observation at the end of seven hours. At the end of twenty-two hours A had no traces of Sudan III, B still had six and C had ten individuals with food.

This early extrusion of indigestible substances is considered in more detail in connection with chinese ink in the following.¹¹

Experiment XXV. Two sets of forty-eight individuals each were fed, one with cold-ether-extracted yolk, the other with chinese ink. Examination of the cell content was made at hour intervals as indicated by numbers on the basal abscissa. Ordinates indicate the number of individuals which had extruded all the ink content in the time indicated (curve A). In curve B the ordinates represent the number of individuals fed extracted yolk in which yolk had disappeared at the end of the time indicated by the abscissa. Chinese ink in suspension is much more readily eaten than carbon or aluminium and is therefore more convenient. This is to be explained by the fact that there are present mucilaginous soluble substances in the chinese ink which serve as agents inducing a more positive feeding reaction and are possibly of some slight food value. The ink was not found to be injurious to the animals. The greater part of the ink is thrown out quite early while slight traces may remain for some time longer. The time during which the ink was retained was taken to end when the last trace

¹¹ In order to obtain satisfactory results with such substances as Sudan III and aluminium in aqueous suspension the adsorbed gases should be driven off before feeding.

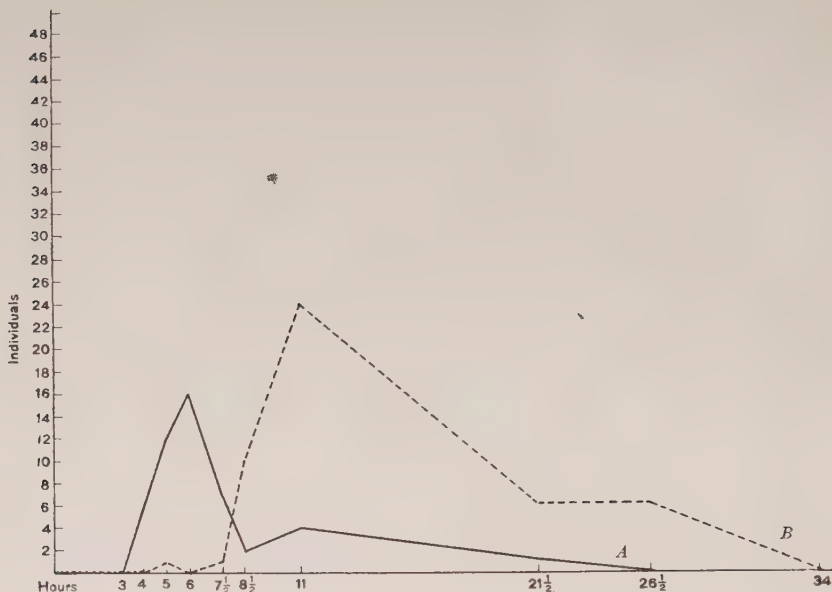


Fig. 5. Experiment XXV. Curve *A* represents the course of extrusion of Chinese ink by forty-eight individuals; curve *B* that of complete digestion of a similar quantity of cold-ether extracted yolk by another set of forty-eight individuals from the same culture.

had been eliminated; curve *A* does not therefore represent the actual time at which the greater part of the ink was extruded but should have its maxima farther to the left than shown. This statement applies to all the extrusion curves which are given.

In figure 5 curve *B*, is that of complete digestion; no extrusion of the extracted yolk took place in this experiment.

After it had been thus shown that ink fed alone to one set of individuals was extruded long before digestion is completed of a similar amount of extracted yolk fed to another set, experiments were carried out to test what the reaction would be if both ink and extracted yolk were fed to the same individuals at the same time. The following two experiments are given to bring out the facts in a quantitative way.

Experiment XXVI. Fifty-four individuals were first fed Chinese ink and immediately afterwards fed with cold-ether-extracted yolk. Two individuals were placed in each watch-glass containing 5 cc. of tap

water and kept in moist chambers. Records were taken noting the presence or total absence of ink and presence or completion of digestion of the extracted yolk at one hour intervals beginning with three and one-half hours up to twelve hours after feeding; three more records were taken at twenty-four, thirty-three and forty-eight hours. The results are expressed in curves in figure 6. Curve *A* represents the extrusion of ink; curve *B*, that of complete digestion of yolk.

It is seen from the relation of the curves that even in this case the ink is extruded before digestion of the extracted yolk is complete, provided that a sufficient quantity of yolk has been eaten.

It was noted that a short time after the ink had been eaten it became assembled into one or several rather definite lumps. This takes place before extrusion. Closer observation further revealed the fact that when ink particles came to be included in vacuoles containing yolk they were not extruded until the food of those vacuoles had been digested, while those which were not included by the yolk vacuoles were very soon extruded. This fact can readily be made out while one follows such experiments as Experiment XXVI above. Bursaria therefore has a power of simultaneous selective extrusion of the contents of different vacuoles as well as a power of selection in the feeding process. This mechanism obviously compensates for the lack of a perfect discriminative and selective function of the oral apparatus.

The results of an experiment (fig. 7) where these facts were taken into account for the purpose of expressing them in a graphic way in curves, is given in the following experiment. A control for comparison was also kept in this case (fig. 7, curve *C*).

Experiment XXVII. Forty-eight individuals were used in each of both the experiment and control. The control (curve *C*, fig. 7) which was fed ink only, shows a sharp early maximum of extrusion from five and one-half to seven and one-half hours after feeding, with three or four individuals retaining traces of ink as long as ten and one-half to twelve hours. Curve *A* represents the extrusion of ink in the forty-eight individuals fed both ink and extracted yolk. It shows two maxima exactly similar to those of curve *A* in Experiment XXVI. Curve *B* (fig. 7) represents the course of complete digestion of the yolk in the same individuals as those of curve *A*. There is only one maximum

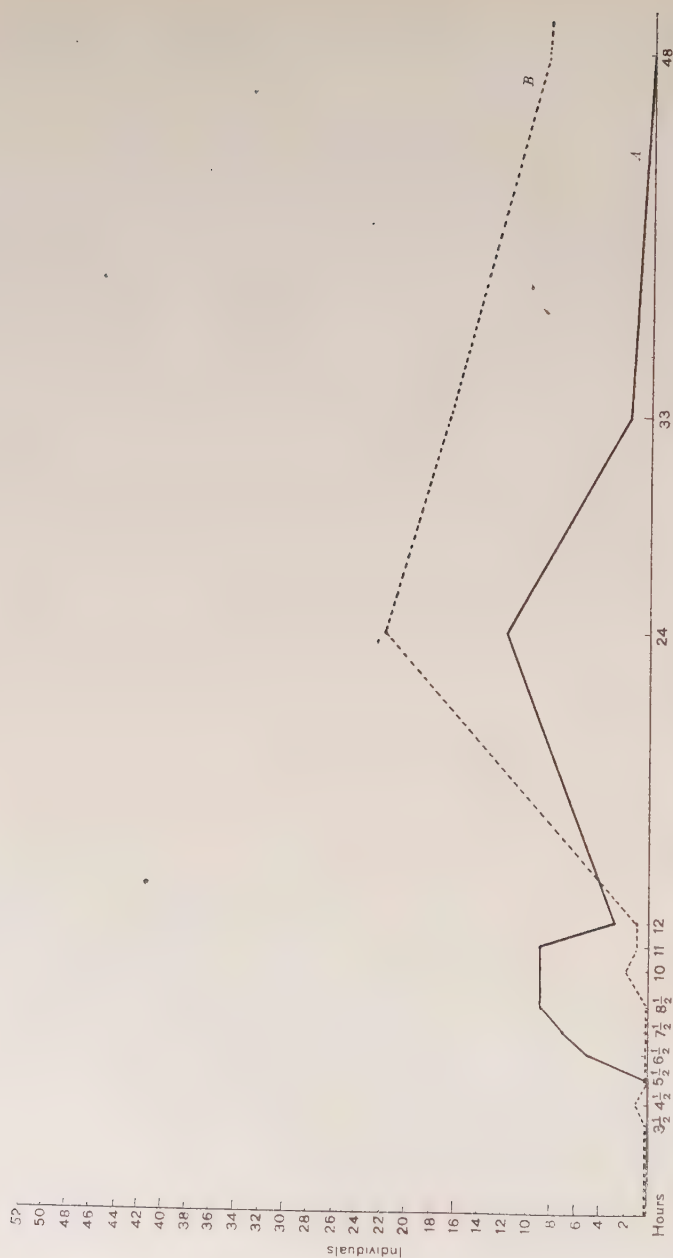


Fig. 6 Experiment XXVI. Curve A represents the course of complete extrusion of Chinese ink fed to fifty-four individuals; it shows two maxima; curve B, course of complete digestion by the same individuals as of curve A; curve B has only one maximum.

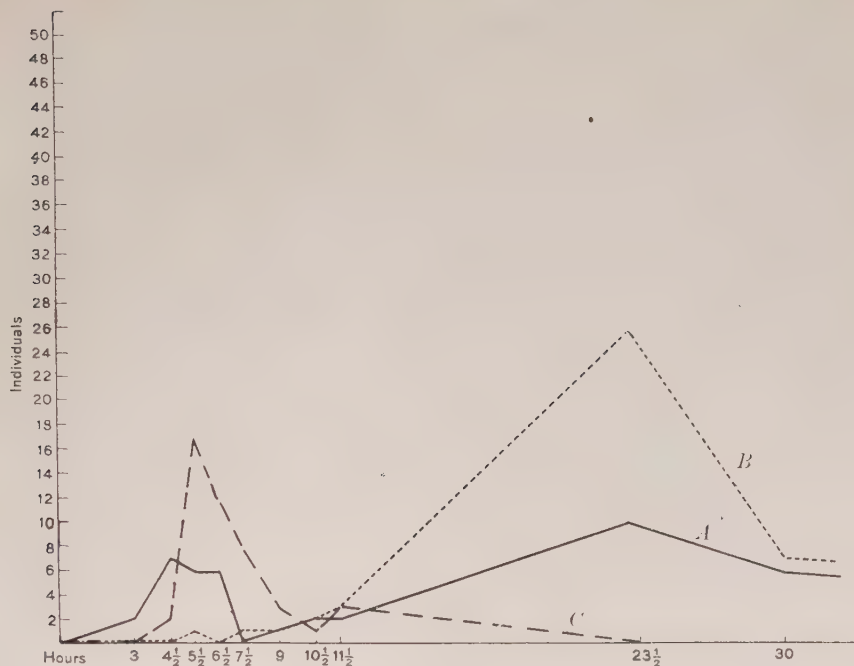


Fig. 7 Experiment XXVII. Curve *A*, course of extrusion of chinese ink showing two maxima (cf. fig. 6, curve *A*); curve *B*, that of complete digestion, by the same individuals used in curve *A*, of extracted yolk; curve *C*, control: course of extrusion of ink by forty-eight individuals fed ink alone.

in this curve, and this comes about eighteen hours later than the first maximum of curve *A* and at about the same time as the second maximum of *B*. The significance of the second maximum in curve *A* is brought out in the following analysis of the two curves *A* and *B*, that is, in a quantitative analysis of the reactions of the forty-eight individuals used in the experiment. All the individuals which at any of the examinations had ink and yolk present in the same vacuole, or vacuoles, were recorded, hence we have a means of dividing the forty-eight individuals into two groups. Group I is made up of those in which, throughout the experiment, yolk and ink were in distinct and separate vacuoles, while Group II, includes those which had during part or all of the time one or more vacuoles which contained both ink and yolk in the same vacuole or vacuoles. Now we have the data which will show just what relation ink and yolk have to each other in the cytoplasm of Bursaria, and what the reaction of the cell is toward each of the two conditions represented by the composition of the vacuoles of the two Groups I and II.



Fig. 8 Analysis of curves A and B of Experiment XXVII, figure 7. *I*, curve A, course of complete extrusion of ink from vacuoles containing only ink; curve B, course of complete digestion of extracted yolk in vacuoles containing only yolk, of the same individuals used in curve A. *II*, Curve A, course of complete extrusion of ink from vacuoles containing both ink and extracted yolk; curve B, course of complete digestion of extracted yolk from the same vacuoles in the same individuals as in curve A.

We may plot the curve of extrusion of ink in group I, and the curve of complete digestion of the same individuals. The curves are given in figure 8, *I*. The same was likewise done for Group II (fig. 8, *II*).

Curve A of figure 8, *I*, (ink and yolk in separate vacuoles) shows now only one early extrusion maximum instead of two. Curve B of figure 8, *I*, is lower (owing to the smaller number of individuals) but exactly similar to B of figure 7. The extrusion of ink from a cell which has its yolk and ink in separate vacuoles is therefore independent of the presence of food and occurs a long time before digestion of the food is completed. The curve of extrusion of ink, A, figure 8, *II*, shows now only one maximum and this corresponds to the second maximum of curve A, figure 7, and is practically identical with curve B of complete digestion. This shows then that whenever ink is included with food in the same vacuole it is retained until its accompanying

food is completely digested, because the maxima of the curves *A* and *B* of *II*, and of *B*, *I*, occur at the same time. We therefore have a demonstration of the selective extrusion among vacuoles as well as of a process of selection in feeding in Bursaria. Similar curves may be worked out for Sudan III or powdered aluminium.

SUMMARY

1. Bursaria has three ways of rejecting solid particles, as shown by the paths over which the particles are passed. These are: (a) the path of total rejection, shown by particles which never enter the oral apparatus; (b) the path of rejection of large particles, this being a retracing in the opposite direction of the path by which they entered; (c) the path of rejection of small particles, which leave the oral pouch by way of the base of the oral sinus and are passed backward over the ventral side of the body (fig. 1).

2. No definite path is followed by the food vacuoles during digestion, and in their passage through the cytoplasm. Residues are eliminated from a small area on the mid-dorsal side of the cell.

3. Grains of fresh hard boiled yolk of hens egg, when prepared as described (page 8), furnishes a good unit of measure of the food taken, and an easy means for determining the factors which come into play in the process of feeding in Bursaria.

4. The amount of food eaten and the rate at which it is eaten depends upon the physiological state of the cell (defined on page 10). This is shown to be true for fresh and for fat-free yolk, and also for indigestible substances such as aluminium, Sudan III, chinese ink, etc.

5. Change in the physiological state of the cell is indicated by the change in the total amount eaten and the rate of feeding, under the same conditions.

6. The rate of feeding is not affected in proportion to the concentration of the yolk suspension.

7. Mechanical stimulation decreases the rate of feeding or inhibits it, roughly in proportion to the degree of stimulation.

8. Rise in temperature increases the rate of feeding on yolk.

9. Continuous action of white light of high intensity had no detectable effect upon feeding on yolk.

10. Feeding may continue during stimulation by a direct electric current of sufficient intensity to control the direction of movement of the organism.

11. *Bursaria* can discriminate between and select non-toxic grains of yolk from among toxic ones. Whether or not *Bursaria* will eat yolk grains that have adsorbed a soluble substance depends upon (a) the steepness of the effective concentration gradient of the dye, between the grain and the non-toxic medium; and this in turn depends upon the amount of dye adsorbed which is subject to a reversible adsorption; (b) the specific chemical properties ('taste?') of the substance adsorbed.

12. There are strong reasons for believing that different parts of the cell are affected unequally by certain toxic substances, and that these may have a specific action upon the selection mechanism, causing a more definite rejecting reaction.

13. Yolk which has adsorbed a substance which is insoluble in water (Sudan III) is eaten as readily as fresh unstained yolk.

14. *Bursaria* has the power of selective extrusion among vacuoles each containing different substances eaten at the same time; vacuoles containing indigestible substances are soon extruded, while those containing food are retained. If fat-free yolk is present in the same vacuole along with the indigestible substance, then the latter is retained until digestion of the enclosed yolk has run its usual course.

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BIOGRAPHY

I, Elmer J. Lund, second son of Cecelia and Peter A. Lund, was born December 13, 1884, near Springfield, Redwood County, Minnesota. My early education was obtained in the public Schools of Olivia, Renville County, Minnesota, and I graduated from the high school at that place in 1906. In the fall of that year I entered Hamline University, where I graduated with the degree of Bachelor of Philosophy, in 1910. During the summer of 1910 I was a member of the Zoölogical Expedition of the Johns Hopkins University to Jamaica. After returning I entered the Johns Hopkins University as a graduate student in Zoölogy, with Botany and Physical Chemistry as subordinate subjects. During my first year I was also student assistant in Zoölogy. In the summer of 1911 I carried on research in Zoölogy, and studied Physics, at the University of Chicago. I published in the *Journal of Experimental Zoölogy* for 1911 a paper entitled "On the Structure, Physiology and Use of Luminous Organs, with Special Reference to the Lampyridae."

During the years 1911-1914 I have held the Adam T. Bruce Fellowship in Biology at the Johns Hopkins University. In the summers of 1912 and 1913 I have been instructor in Zoölogy at the Marine Biological Laboratory at Woods Hole, Massachusetts.

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